Novel T7-modified pH-responsive targeted nanosystem for co-delivery of docetaxel and curcumin in the treatment of esophageal cancer

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Figure Captions:

Figure S1: TfR expression in ESCC. High TfR expression in ESCC was demonstrated by qPCR (A) and verified by flow cytometry (B, C). * $P < 0.05$.

Figure S2: Characterization of nanoparticles. The successful synthesis of T7-NP was demonstrated by ^{1}H NMR.

Figure S3: pH stimuli-responsive drug-release *in vitro*. Cumulative drug-release was measured by UV spectroscopy (UV-S) and HPLC (A-B).

Figure S4: Biocompatibility of nanoparticles. Cells were pretreated with different nanoparticles (NP and T7-NP) at different concentrations. CCK-8 assay demonstrated that there was no damage to cells by nanoparticles at high concentration (A, B).

Figure S5: Simulation of anti-tumor effect by 3D tumorsphere. 3D tumorspheres were prepared *in vitro* to simulate tumors and treated with various formulations (PBS, DTX, NP-D, NP-DC, T7-NP-D and T7-NP-DC). Apoptosis was determined by optical microscopy (A, B).

Figure S6: Synergistic anti-tumor efficacy of nanomedicines in ESCC. Cells (KYSE510) were incubated with different treatments (DTX, CUR, NP, NP-T7, NP-D, NP-C, NP-DC, T7-NP-D, T7-NP-C and T7-NP-DC) for 48 h. Cell viability was then measured by flow cytometry (A, B) and CCK-8 assay (C). * $P < 0.05$, ** $P < 0.01$.

Figure S7: Preferential cellular uptake of T7-modified nanoparticles. Cells (KYSE510) in good condition were pretreated with FITC-labeled nanoparticles composed of different treatments. The fluorescence intensity in cells was then observed using a confocal microscope. Cellular uptake of T7-decorated nanoparticles was superior to that of nanoparticles without T7-decoration..

Figure S8: Biosafety of nanomedicines in respect of main organ function and hematopoietic function of bone marrow.

Table S1: Combination index (CI)of different drug formulations in KYSE510.

DTX (ng/ml)	DTX:CUR 2:1	DTX:CUR 1:1	DTX:CUR 1:2
	CI	CI	CI
0.0625	0.17033	0.16518	0.10746
0.125	0.22973	0.25660	0.18218
0.25	0.44085	0.16610	0.15473
0.5	0.33826	0.22142	0.19966
1	0.44548	0.37128	0.36934
2	0.71650	0.60316	0.54217
4	1.25438	0.78015	0.71996
8	1.61885	1.40310	0.99193

Table S2: IC_{50} values of different DTX formulations in KYSE150 and KYSE510.

	IC50 DTX(ng/ml)			
Formulations	KYSE150		KYSE510	
Free DTX	13.49±2.57		3.07±0.62	
NP-DTX	12.066±1.33		2.64±0.26	
NP-DTX/CUR	9.47±0.55	**	2.38±0.94	*
T7-NP-DTX	5.57±0.37	***	1.36±0.12	***
T7- NP-DTX/CUR	3.15±0.78	***	0.43±0.11	***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$

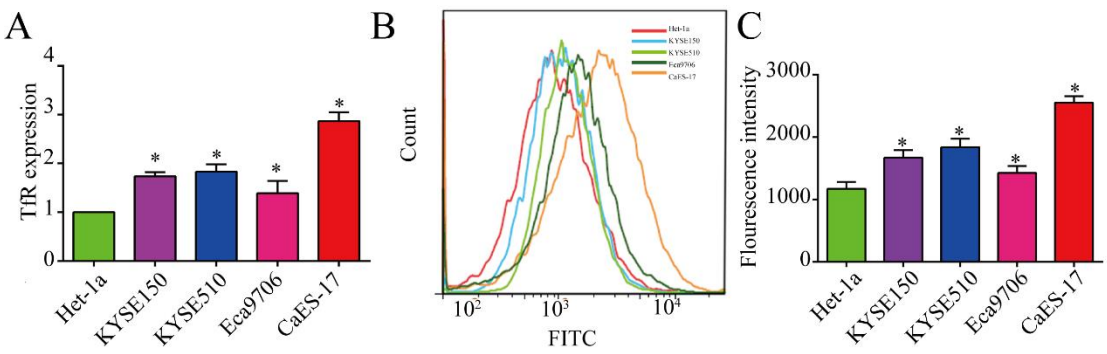
A
TfR expression
4
3
2
1
0
*
*
*
*
Het-1a
KYSE150
KYSE510
Eca9706
CaES-17
B
Count
Het-1a
KYSE150
KYSE510
Eca9706
CaES-17
10^2
10^3
10^4
FITC
C
Flourescence intensity
3000
2000
1000
0
*
*
*
*
Het-1a
KYSE150
KYSE510
Eca9706
CaES-17

Figure S1

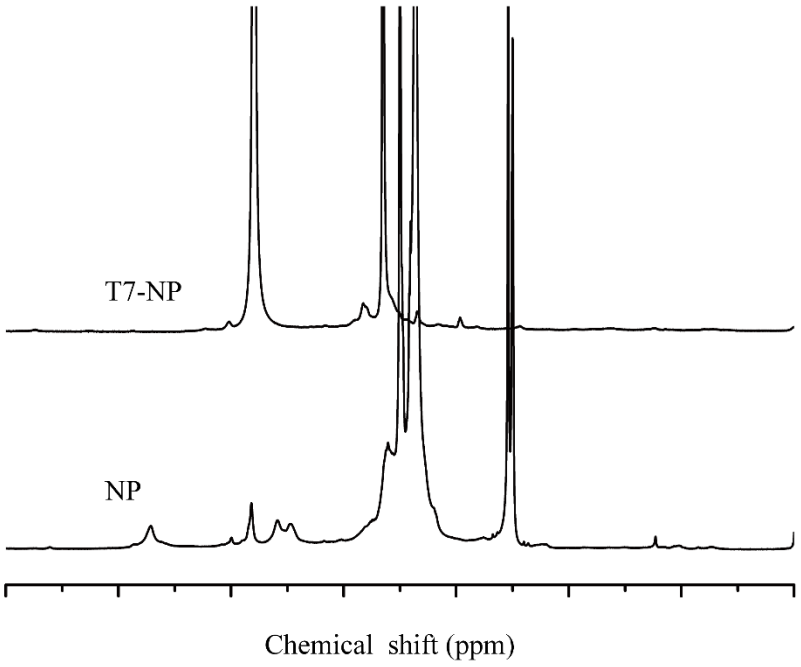

Figure S2

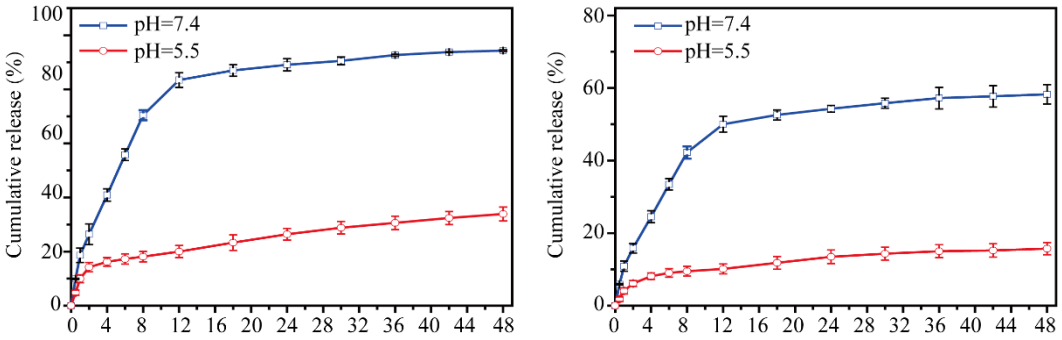
pH=7.4
pH=5.5
Cumulative release (%)
0
20
40
60
80
100
0 4 8 12 16 20 24 28 32 36 40 44 48
pH=7.4
pH=5.5
Cumulative release (%)
0
20
40
60
80
0 4 8 12 16 20 24 28 32 36 40 44 48

Figure S3

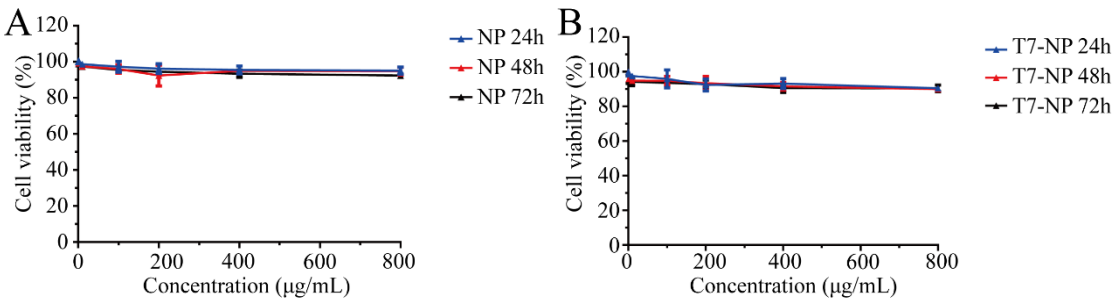
A
Cell viability (%)
120
100
80
60
40
20
0
0
200
400
600
800
Concentration (μg/mL)
NP 24h
NP 48h
NP 72h
B
Cell viability (%)
120
100
80
60
40
20
0
0
200
400
600
800
Concentration (μg/mL)
T7-NP 24h
T7-NP 48h
T7-NP 72h

Figure S4

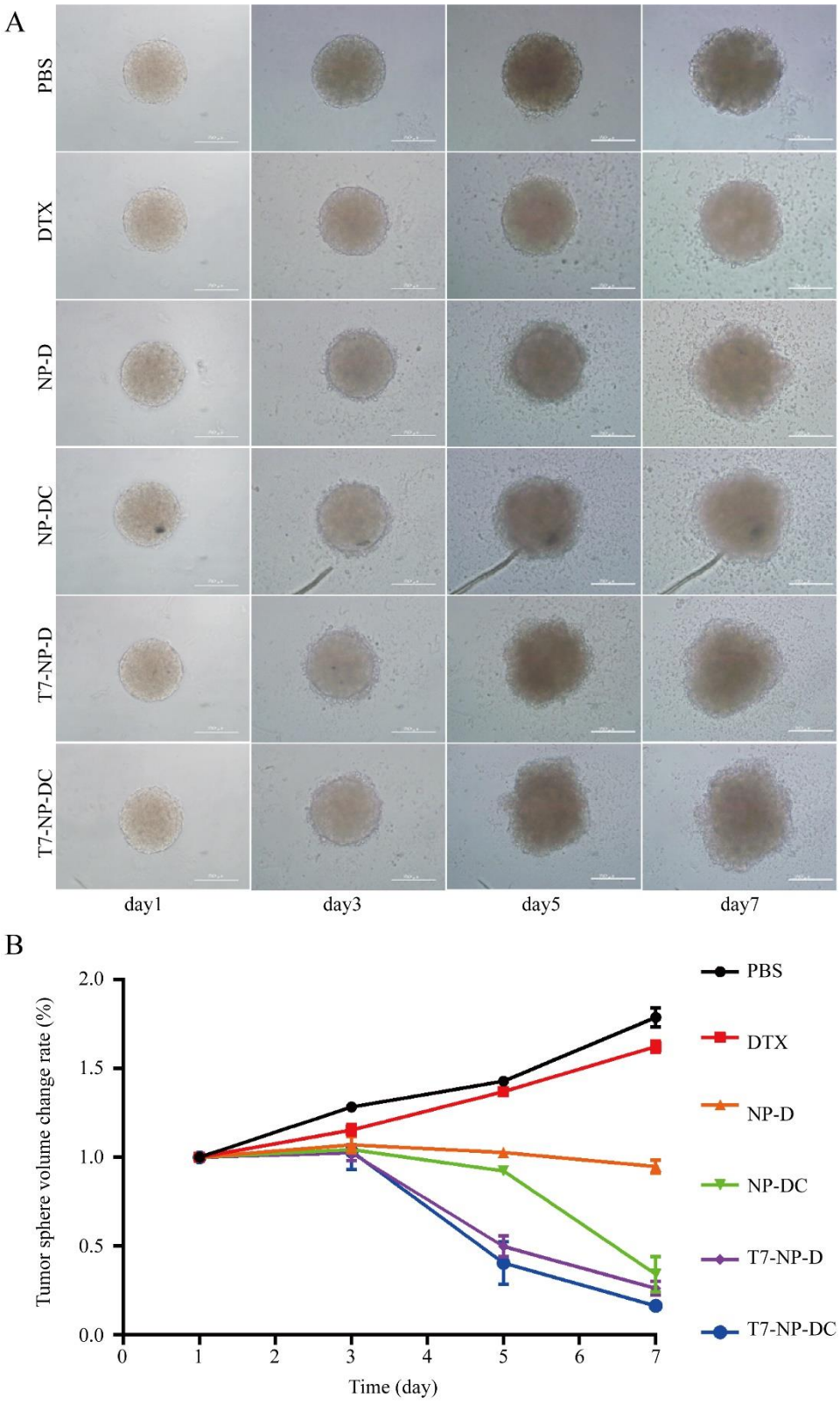
A
PBS
DTX
NP-D
NP-DC
T7-NP-D
T7-NP-DC
day1
day3
day5
day7
B
Tumor sphere volume change rate (%)
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
Time (day)
PBS
DTX
NP-D
NP-DC
T7-NP-D
T7-NP-DC

Figure S5

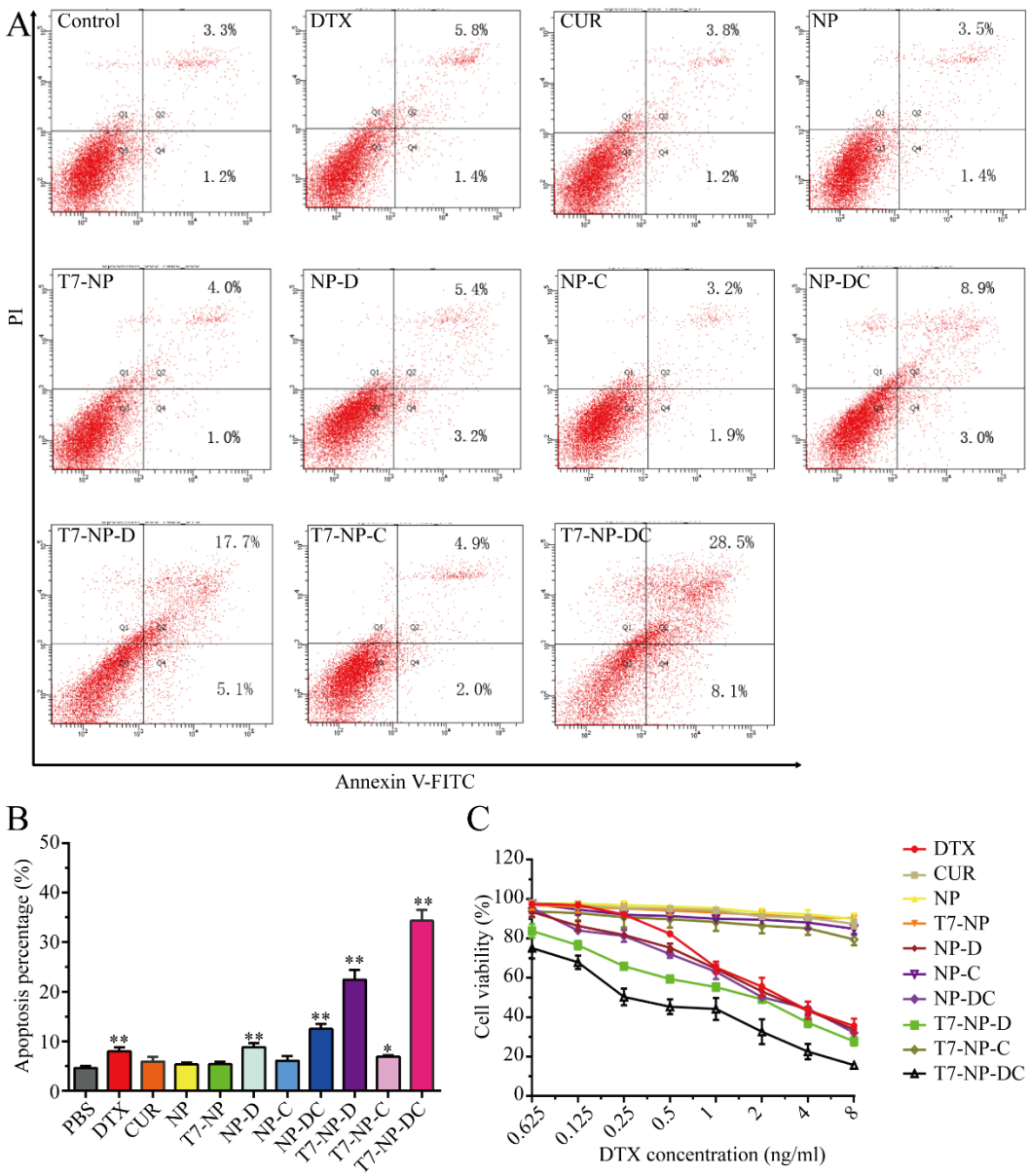
A
Control
3.3%
1.2%
DTX
5.8%
1.4%
CUR
3.8%
1.2%
NP
3.5%
1.4%
T7-NP
4.0%
1.0%
NP-D
5.4%
3.2%
NP-C
3.2%
1.9%
NP-DC
8.9%
3.0%
T7-NP-D
17.7%
5.1%
T7-NP-C
4.9%
2.0%
T7-NP-DC
28.5%
8.1%
PI
Annexin V-FITC
B
Apoptosis percentage (%)
PBS
DTX
CUR
NP
T7-NP
NP-D
NP-C
NP-DC
T7-NP-D
T7-NP-C
T7-NP-DC
C
Cell viability (%)
DTX concentration (ng/ml)
DTX
CUR
NP
T7-NP
NP-D
NP-C
NP-DC
T7-NP-D
T7-NP-C
T7-NP-DC

Figure S6

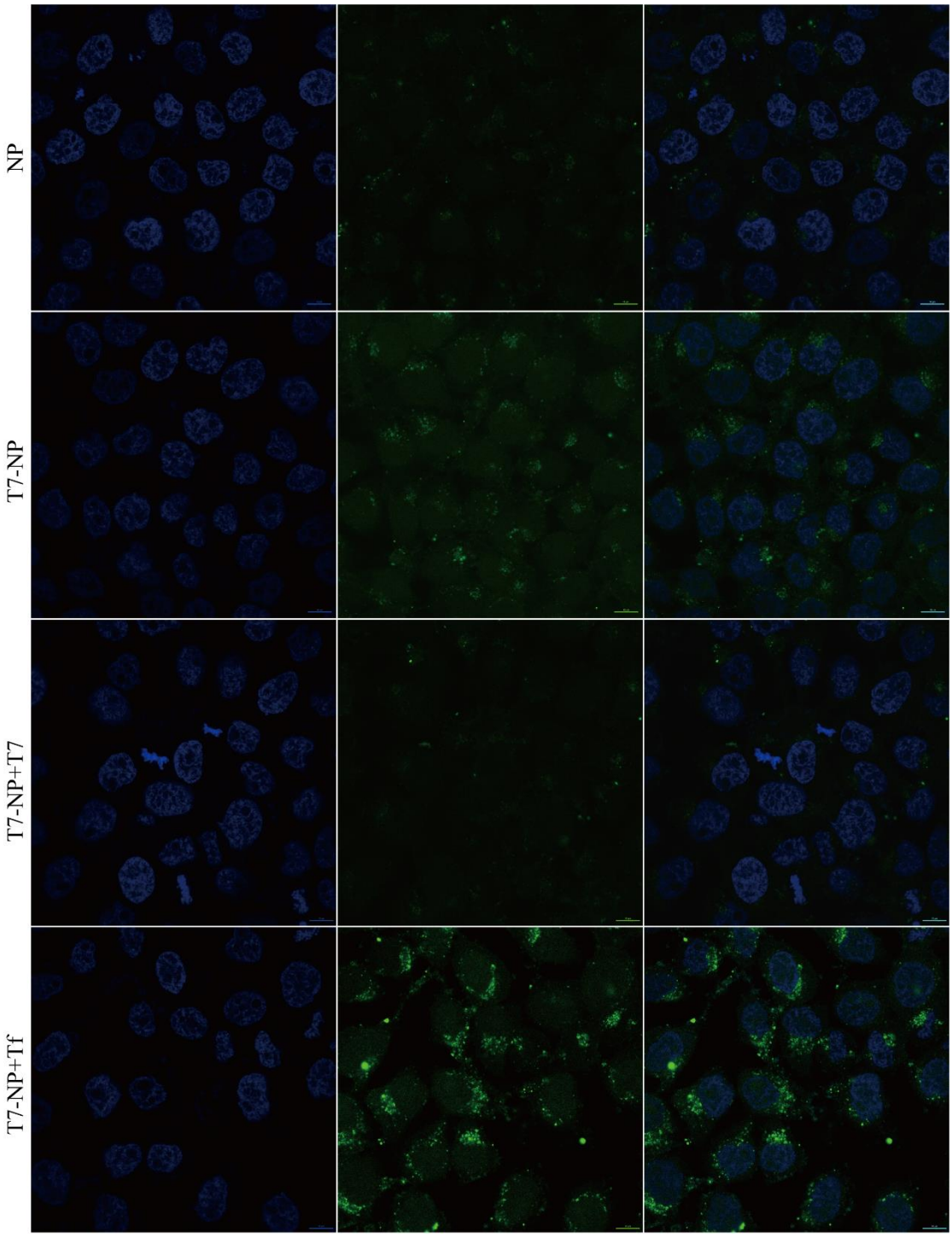

Figure S7

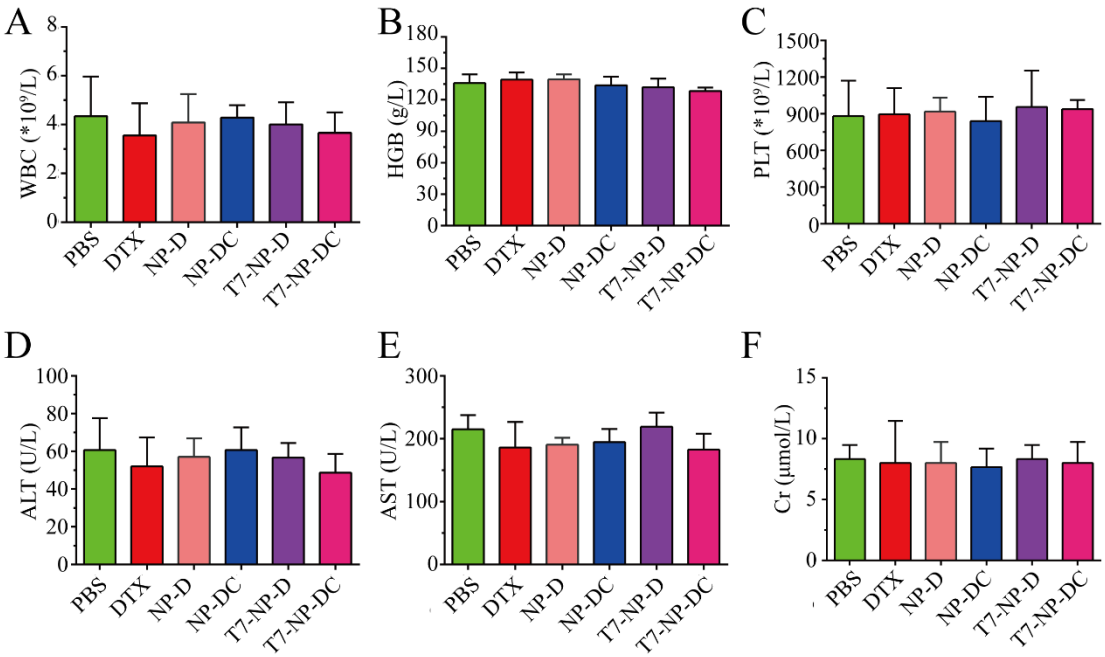
A
WBC (*10⁹/L)
0
2
4
6
8
B
HGB (g/L)
0
30
60
90
120
150
180
C
PLT (*10⁹/L)
0
300
600
900
1200
1500
D
ALT (U/L)
0
20
40
60
80
100
E
AST (U/L)
0
100
200
300
F
Cr (μmol/L)
0
5
10
15
PBS
DTX
NP-D
NP-DC
T7-NP-D
T7-NP-DC

Figure S8